AD 299 116

LINDE COMPANY
DIVISION OF UNION CARBIDE CORPORATION
TONAWANDA LABORATORIES
TONAWANDA, N.Y.

PROGRESS REPORT

RESEARCH ON PROCEDURES FOR THE

LOW-TEMPERATURE PRESERVATION OF BLOOD

XIII

CONTRACT NO. NONR-3003(00)

Prepared for

Office of Naval Research
Department of the Navy

Submitted by

A. P. Rinfret
Principal Investigator

Research Laboratory
Linde Company
Division of Union Carbide Corporation

March 20, 1963

Copy No. 47

PROGRESS REPORT

RESEARCH ON PROCEDURES FOR THE

LOW-TEMPERATURE PRESERVATION OF BLOOD

XIII

Abstract

Optimal processing conditions for freezing and thawing whole blood with 7% w/v PVP (Plasdone C) have resulted in reproducible recovery of 97% of the red cells immediately after thawing. Infusion of such preparations in quantities up to one pint have shown an average of 80% of the red cells survive in the circulation for 24 hr. The thawed preparation has 3% of the hemoglobin and 40% of the potassium in the plasma, and it contains a precipitate of β-lipoprotein that is soluble in isotonic saline and plasma.

Protein precipitate can be minimized by suspending (1 vol. - 1 vol) packed red cells in a solution of 14% PVP + 3% human serum albumin before freezing. The same post-thaw recovery, 97%, results, but 24-hr. survival averages 88% instead of the 80% for whole blood.

Old and young cells lyse to the same extent during freezing and thawing, and both are more susceptible to osmotic lysis after thawing than before freezing.

TABLE OF CONTENTS

Researchers Participating in the Experimental Program Summarized in This Report:

Linde:
- E.O. Brimm, Ph.D.
- R.G. Buchheit
- C.W. Cowley
- G.F. Doebbler, Ph.D.
- R.F. Dwyer
- J.A. Lawrie
- C.H. Nuermberger
- A.P. Rinfret, Ph.D. (principal investigator)
- H.D. Robbins
- A.W. Rowe, Ph.D.
- R.R. Sakaida, Ph.D.
- J.A. Sawdye
- H.R. Schreiner, Ph.D.
- A.J. Short

VA Hospital, Buffalo
- M.L. Bloom, M.D.
- T.M. Bow
- C D Bull, M D.

Roswell Park Memorial Institute
- R.S. Kibler, M.D.

SCOPE OF REPORT:

This report summarizes the current status of processes for the low-temperature preservation of red cell suspensions suitable for infusion immediately after thawing; it is based largely on research done in the period June 1962 to February 1963, under Contract Nonr-3003(00).

I. INTRODUCTION:

Our goal has been the development of a process for preserving a suspension of red cells by freezing, with subsequent long-term storage at low temperature. Upon thawing, the product must be suitable for infusion without any separation or washing steps. To be acceptable the cells must remain unaltered to a high degree during freezing and thawing and must be stable in the circulation on infusion, and any substance added to give such a stable cell population must be physiologically acceptable in the concentrations achieved on infusion.

Experimental results given in previous Progress Reports have led us to the following generalizations:

1. A high recovery of intact cells is achieved only when red cells are frozen in the presence of a medium containing a minimum of about 10% by weight of an added substance.

2. When the additive is capable of penetrating the red cell membrane, there is marked lysis of cells on infusion; osmotically stable cell suspensions are recovered only with additives that do not enter the cell.

3. Carefully controlled, rapid rates of heat transfer are necessary during freezing and thawing to get a high recovery of stable cells in the presence of extra-cellular additives.

A red cell preparation with high recovery of intact cells on thawing and high stability on infusion, with the potential of fulfilling the objectives of the project, was found to be obtained by addition of polyvinylpyrrolidone (PVP) to blood (plus anticoagulant) to give a 7% w/v concentration of PVP followed by rapid freezing and rapid thawing. This has been given the designation of Process III in Progress Report XI. The current report covers our further study of the utility of the thawed product and the practicality of the processing, together with a discussion of the modifications that have been considered desirable at this time.

II. BACKGROUND TO THE PRESENT RESEARCH ON THE PVP-WHOLE BLOOD SYSTEM:

In order to meet the important requirement that thawed blood be transfusible without post-thaw processing, it was found necessary to use an additive that does not enter the cell. Red cells equilibrated with concentrations that afford protection of glucose, glycerol, or dimethylsulfoxide, lyse immediately and completely on infusion. A principal problem, then, was the selection of an additive that does not enter the red cell, affords protection during freezing and thawing, and is pharmacologically acceptable on infusion. Of the many materials tried, PVP appeared most promising, for it resulted in the highest recovery of intact cells capable of surviving to a high degree on infusion, and had been used for many years as a plasma volume expander without manifestation of acute effects. Dextran and human serum albumin, although physiologically acceptable, did not give the same degree of protection.

Having established that the PVP-whole blood system was the most promising, we have concentrated our efforts on determining the optimum processing conditions and ensuring reproducibility of operation, demonstrating the biological functionality of processed red cells, and searching for possible detrimental effects on blood of PVP and of freezing and thawing.

Among the matters studied have been:

1. Red Cell Stability
   - a. in vitro
   - b. in vivo
2. Red Cell Function
   - a. Oxygen dissociation
   - b. Exchange transfusions
3. PVP-Protein Interaction
4. Processing Variables
   - a. PVP concentration
   - b. PVP molecular weight distribution
   - c. PVP-albumin medium
   - d. Freezing and thawing conditions

Details of these studies are given in the following sections.

III. PVP-WHOLE BLOOD:

A. Description of Process:

In the process being considered currently, 420 cc. of blood is drawn into 160 cc. of a solution containing 43% ACD-A and 26.5% PVP (Plasdone C) to give a total volume of 580 cc. The container is aluminum, is corrugated to give a high ratio of surface to volume, and has a volume of 1000 cc.

The container of blood is coated with a thin (0.004 in.) layer of PVP and then immersed into liquid nitrogen while being shaken. This results in the formation of a shell of frozen blood 3-9 mm. thick. After immersion for 120 sec. the container is stored at -170°C. or below until needed.

Thawing is accomplished by shaking the container in a bath of water at 45°C. for about 75 sec., after which it is immediately withdrawn and stored at 4°C. until administered. A newly designed bracket has decreased thawing time to about 62 sec., but the effect of the shorter thawing time on recovery and stability has not been evaluated.

B. Stability of Cells.

1. In Vitro Measurements:

a. Recovery:

When red cells are frozen and thawed in the presence of plasma plus PVP, some of the cells lyse. The extent of lysis is determined by the concentration of PVP and the thermal regimen experienced by the cells. Under best conditions 97% of the cells are reproducibly recovered intact, as measured by the loss of hemoglobin from cells. Potassium ion loss is greater than that resulting from lysis alone and is in the order of that of 21-day-old bank blood, 22 meq./l. vs. 23 meq./l. for 21-day-old blood.[1] Direct infusion of such a product introduces free hemoglobin and potassium ion into the circulation in addition to the PVP required for protection. This is discussed further in the next section.

b. Resuspension Stability:

Loss of hemoglobin and potassium from cells is a direct manifestation of cell damage. There is additional damage

which is shown by the loss of cells on infusion and which can be demonstrated in vitro by diluting cells with isotonic saline. There is additional hemolysis, the extent of which can give an indication of the degree of trauma experienced by the cells. Preparations that give 97% recovery of intact cells on thawing may lose an additional 5 to 17% of the cells on resuspension in forty-fold excess (or greater) of isotonic saline (Fig. 1). Those that show the largest additional hemolysis have the poorest survival on infusion, and those with small loss may, or may not, have good survival.

It appears, then, that in vitro tests do not distinguish unequivocally between preparations that will show the best in vivo survival and those that are poorer, but if they indicate a preparation is less stable, lower survivals will be obtained with it.

c. Effect of Age of Cells on Stability:

A question of fundamental interest regarding freeze-thaw damage to erythrocytes is whether such destruction is related to in vivo age of the cells. Older erythrocytes are physically and biochemically different from young cells and more susceptible to osmotic lysis.[2] It is entirely possible that the older cells which are soon to be destined for removal from circulation are those which are particularly susceptible to the rigors of low-temperature processing of blood. If this were so, it might be possible to minimize hemolysis by appropriate treatment of this fraction.

A direct approach to this question is to label selectively one portion of the population and determine its fate. To this end, radioactive iron-59 was injected into rabbits for the purpose of labeling the younger population of the cells since only the newly formed erythrocytes incorporate the iron. Samples of the blood were taken 5 to 8 days after iron-59 injection and frozen after addition of PVP to give a 7% concentration. In the prefreeze and post-thaw samples the distribution of hemoglobin and radioactivity between plasma and cells was determined.

It is apparent from the results in Table I that equal fractions of the total hemoglobin and radioactivity were found to have been released into the supernatant after freezing, indicating no difference in the level of hemolytic damage between the young cells and the rest of the population.

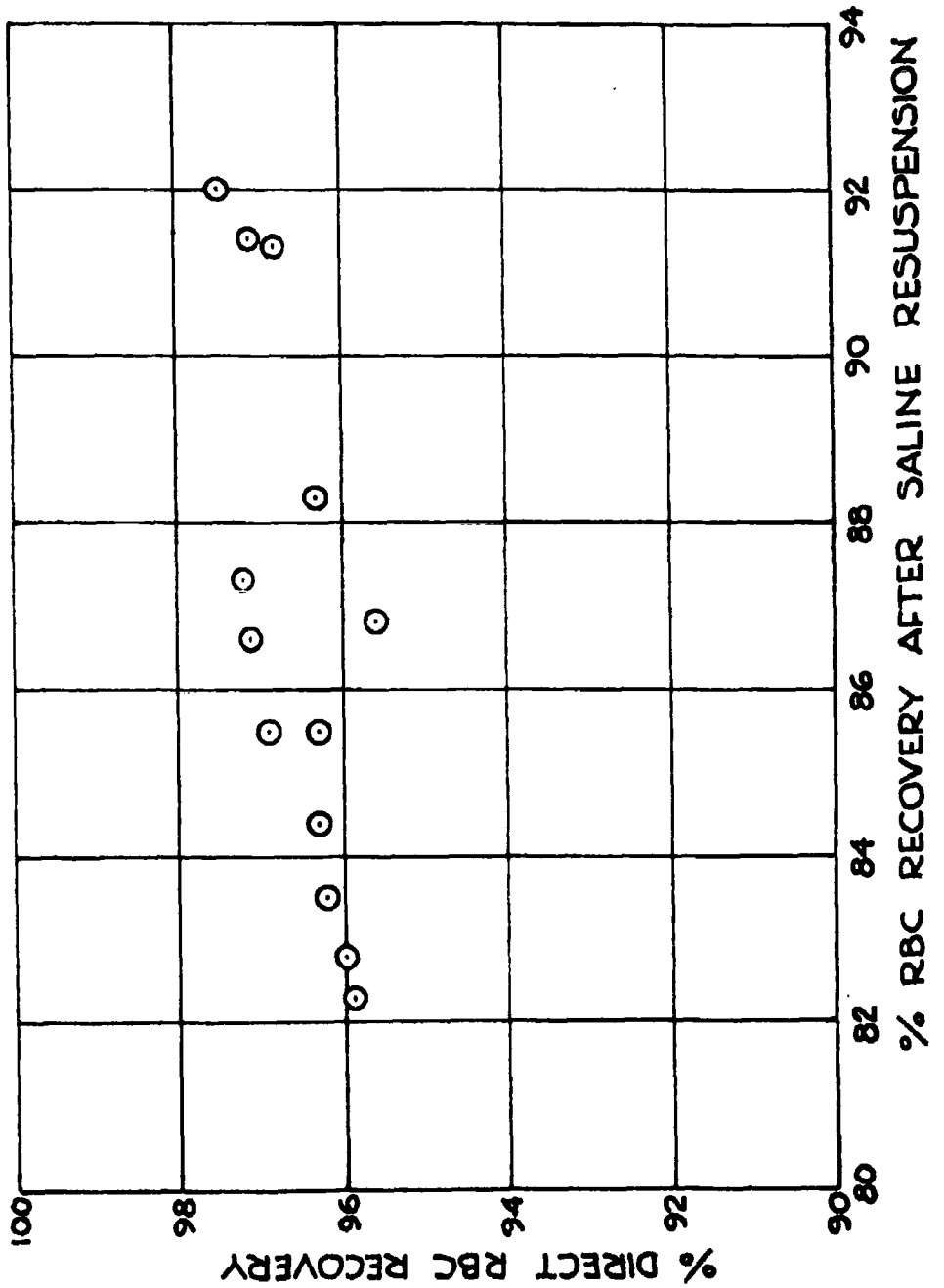
% DIRECT RBC RECOVERY
100
98
96
94
92
90
% RBC RECOVERY AFTER SALINE RESUSPENSION
80
82
84
86
88
90
92
94

Fig. 1

TABLE I

Freeze-Thaw Hemolysis of Process III[a]

Rabbit Blood--Effect of Red Cell Age

as Determined by Fe^{59} Labeling

In Vivo Age of Cells After Fe^{59}Tag[b] (Days)	Prefreeze		Post-Thaw	
	Entire Population[c] (as % Free Hemoglobin)	Young Cells[d] (as % Free Radioactivity	Entire Population[c] (as % Free Hemoglobin)	Young Cells[d] (as % Free Radioactivity)
8	<0.3	0.1	2.4 2.9	2.4 2.8
5	<0.2	0.2	3.8 4.1	3.5 3.6
5	0.2	0.3	2.0 2.1	2.4 2.3

a Process - Blood (4 volumes) collected in ACD-B (1 volume) diluted (4:1) with 35% Plasdone "C" in 0.05 M NaCl. Blood + PVP frozen in BFT-19110 containers coated with glycerol-Santocel using BPU-1 (200 cpm agitation). Thawing by agitation in 45°C. water bath.

b Fe^{59}Tagging - Young red blood cells selectively labeled in vivo by incorporation of Fe^{59} (50 μc as ferrous citrate injected intravenously)

c Entire Population - % free hemoglobin calculated using total and supernatant hemoglobin and hematocrit.

d Young Cells - % free radioactivity calculated using total and supernatant radioactivity and hematocrit.

After freezing and thawing, the red cells are usually resuspended in saline for the EDP test to determine subhemalytic damage which manifests itself as hemolysis of apparently intact cells. From Table II it may be seen that both before and after freezing the old cells are more susceptible to osmotic lysis than are the younger cells as shown by osmotic fragility measurements.

TABLE II

Osmotic Fragility of Rabbit Blood Before and After Freezing by Process III[a] -- Effect of Red Cell Age as Determined by Fe^{59} Labeling

NaCl Conc. (%)	Prefreeze		Post-Thaw	
	Entire Population[c] (as % Free Hemoglobin)	Young Cells[d] (as % Free Radioactivity)	Entire Population[c] (as % Free Hemoglobin)	Young Cells[d] (as % Free Radioactivity)
0.85	0.3	0.3	15.9	10.0
0.7	0.3	0.3	17.9	11.0
0.6	3.9	0.5	32.1	14.8
0.55	28.8	7.8	60.3	34.2
0.5	74.0	55.3	83.0	67.8
0.45	94.3	93.0	93.0	87.7
0.4	98	99	96	96
0.3	99	99	97	97
0.2	100	100	99	98
0.0	100	100	100	100

a Process III - Procedures are the same as outlined in Table I. Results are based on an average of three rabbits.

b Fe^{59} Labeling - Young cells selectively labeled in vivo by incorporation with Fe^{59} (μc as ferrous citrate injected intravenously).

c Entire Population - % free hemoglobin calculated using total and supernatant hemoglobin and hematocrit.

d Young Cells - % free radioactivity calculated using total and supernatant radioactivity and hematocrit.

2. In Vivo Measurements:

a. Survival After Infusion:

The stability of processed cells in the circulation has been measured by the Cr^{51}-Cr^{51} double tagging procedure described in Progress Report XII. The recipient's red cell volume is determined from the dilution of a known volume of Cr^{51}-tagged unfrozen, autologous blood. Tagged frozen and thawed blood is then infused for measurement of survival.

Using the optimal combination of processing parameters--PVP concentration, freezing and thawing rates, and container size and geometry--an average of 80% of the cells intact after thawing have been found circulating 24 hr. after transfusion. Representative data are given in Table III.

TABLE III

Average In Vivo Survival of Frozen and Thawed Blood

Whole Blood + ACD + 7% w/v PVP

Blood Volume (ml.)	Container Size (ml.)	Tests	Recovery	Saline Stability	Survival 30 min.	24 hr.	48 hr.	72 hr.
50	110	6	96 ± 1	87± 2	94± 4	79± 4	-	-
250	500	17	97 ± 1	89± 2	93± 8	82-7	80± 8	75± 7
450	1000	21	95.6± 0.5	83± 2.7	86± 1.7	79± 9	71± 7	67± 7

Most of loss of cells occurs in the first few hours after transfusion (Fig. 2).

b. Mechanism of Loss of Red Cells Immediately Following Infusion:

When intact cells disappear after infusion, they may be lysed in the circulation or may be removed in the RE system. Evidence against any marked lysis is given in Table IV, which tabulates data obtained in one-pint transfusions. Column 8 represents the percentage of the

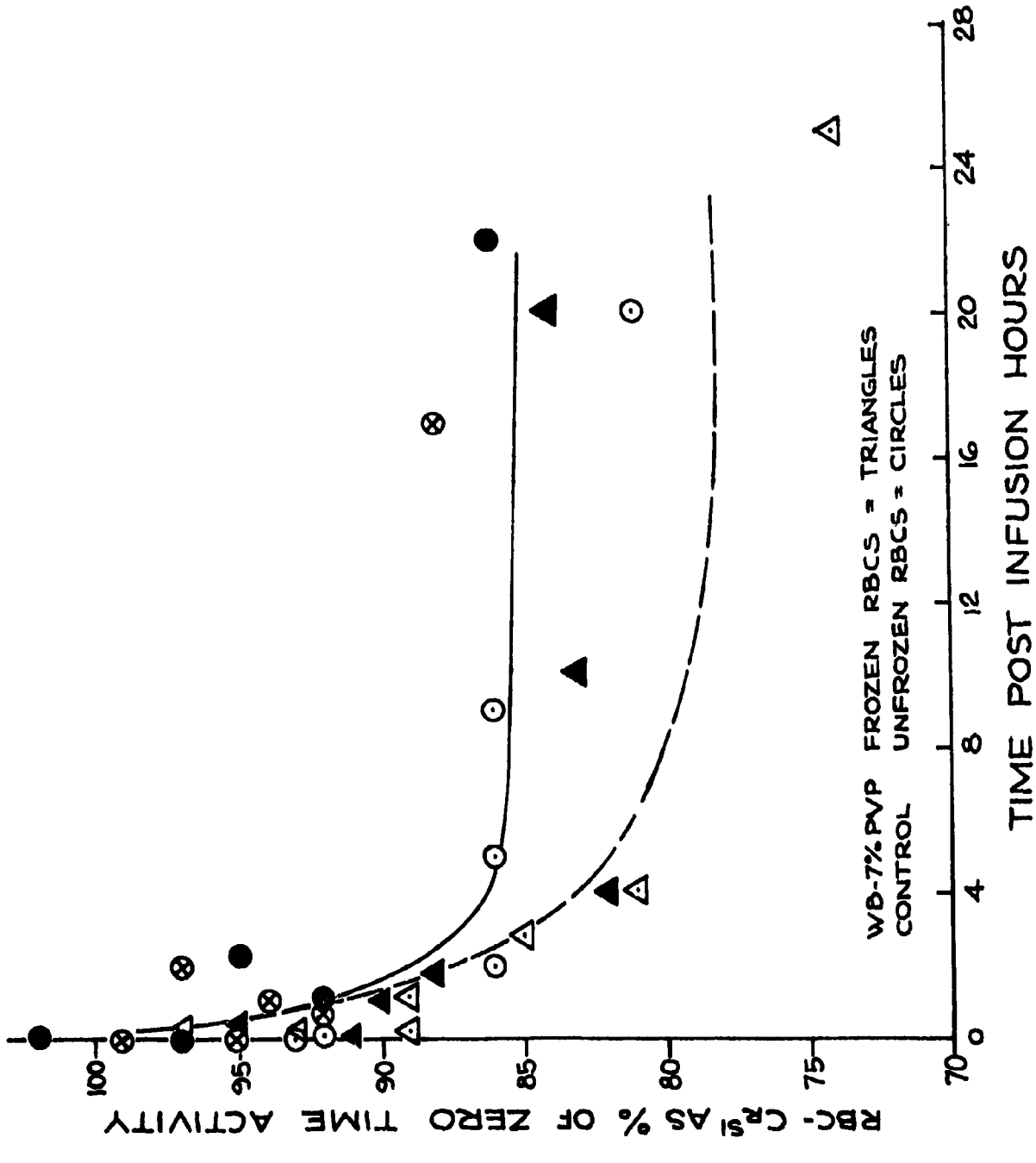
RBC- Cr^{51} AS % OF ZERO TIME ACTIVITY
100
95
90
85
80
75
70
0
4
8
12
16
20
24
28
TIME POST INFUSION HOURS
WB-7% PVP FROZEN RBCS = TRIANGLES
CONTROL UNFROZEN RBCS = CIRCLES

Fig. 2

TABLE IV

One-Pint Transfusions

Whole Blood + PVP

Normal Recovery, %	Saline Yield (1:100), %	Volume Transfused, ml.	Amt. of Hemoglobin Transfused, g.	Post-Transfusion Plasma Vol., ml.	Plasma Hemoglobin 30-Min. Post-Trans., mg-%	Total Circulating Hemoglobin 30-Min. Post-Trans., g.	$100\left(1-\frac{\text{Hbg circ.}}{\text{Hgb infused}}\right)$	[(1) - (8)]
96.8	88.7	386	35.5	2558	68	1.74	95.1	1.7
95.5	84.1	390	38.2	2678	91	2.44	93.6	1.9
95.6	86.8	381	34.2	2740	49	1.34	96.1	~ 0
96.5	88.9	377	34.3	2698	59	1.59	95.4	1.1
97.3	90.3	382	33.6	3048	30	0.91	97.3	0
97.1	89.9	0	-	-	-	-	-	-
97.5	92.1	379	33.4	1904	35	0.67	98.0	~ 0
97.1	90.8	383	39.1	2333	48	1.12	97.1	0
96.2	87.5	0	-	-	-	-	-	-
97.3	89.4	269	24.2	2197	37	0.81	96.7	0.6
96.5	85.0	395	38.0	2639	95	2.50	93.4	3.1
95.5	85.7	386	38.2	2819	84	2.37	93.8	1.7
96.8	86.5	387	37.9	2462	71	1.75	95.4	1.4
96.8	88.5	384	34.9	2601	66	1.72	95.1	1.7
97.2	87.6	384	38.4	2465	70	1.72	95.5	1.7
96.3	84.3	380	36.4	2467	77	1.90	94.8	1.5
95.1	81.6	396	37.6	2860	70	2.00	94.7	0.4
96.3	85.0	391	37.2	1767	87	1.54	95.9	0.4
96.1	85.1	390	41.8	2384	88	2.10	95.0	1.1
96.0	85.7	395	39.9	2327	99	2.30	94.2	1.8
95.8	83.7	396	41.9	2558	100	2.56	93.9	1.9

total infused hemoglobin that is not accounted for as free hemoglobin in the plasma. It is equivalent to the recovery of intact cells after transfusion. Comparison (Column 9) of this value with the recovery of the cells before transfusion (Column 1) shows that stable cell preparations, as indicated by saline resuspension values of 87% or greater (Column 2), have lost less than 2% of their cells by lysis on transfusion. Although there are uncertainties in these measurements it is unlikely that much free hemoglobin has been removed from circulation in this time, for the haptoglobin-binding capacity has not been exceeded.

It would appear, then, that red cells frozen and thawed in the presence of plasma plus PVP are not removed primarily by lysis in the first 30 min. after transfusion when optimal processing conditions are used. A less stable preparation might well lose some of the population by intravascular lysis in the period immediately after transfusion.

c. Biological Functionality of Frozen and Thawed Red Cells:

Not only must processed red cells remain intact in the circulation over their normal life span, but they must carry out their functions to transport oxygen and carbon dioxide. The oxygen dissociation equilibrium was found (Fig. 3) to be identical with that of unfrozen cells. Because the rates of oxygen uptake and release are most significant, exchange transfusions with processed blood were carried out in rabbits as a way of establishing the oxygen-carrying capacity in vivo. Whole rabbit blood with PVP was frozen under the optimum conditions for rabbit blood. After thawing it was transfused into rabbits that had been partly bled or were bled simultaneously with the transfusion. In the course of these transfusions it was discovered that the amount of potassium ion being infused was sufficient to raise the concentration in plasma to a lethal level (10 meq./l.) at the rates of infusion used (6 cc./min.). This was substantiated by infusing blood after passage through a bed of ion exchange resin (Dowex 50) in the sodium form. When this was done a volume of processed blood greater than a normal blood volume could be infused without manifestation of potassium toxicity.

Because of the difficulty of carrying out exchange transfusions with rabbits, the highest degree of exchange with low-potassium blood has been 40%, although 85% exchange has been achieved with blood containing potassium. We have found from earlier work that this is insufficient for evaluation of functionality; rabbits can survive after 90% of their red cells have been removed. This study is continuing, as is an evaluation of potassium leakage from human cells during freezing and thawing.

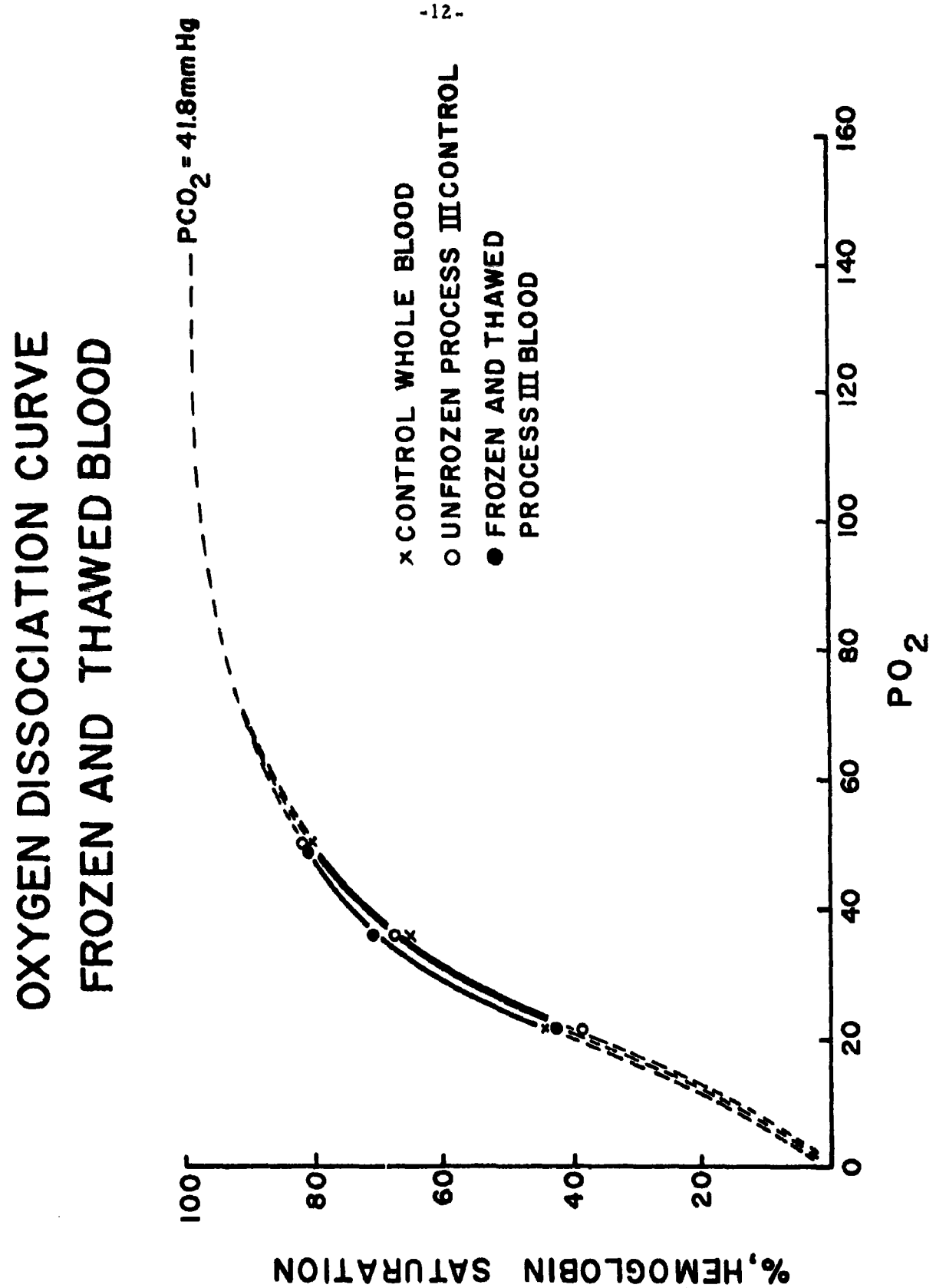
OXYGEN DISSOCIATION CURVE
FROZEN AND THAWED BLOOD
PCO_2 = 41.8mm Hg
x CONTROL WHOLE BLOOD
o UNFROZEN PROCESS III CONTROL
● FROZEN AND THAWED PROCESS III BLOOD
% HEMOGLOBIN SATURATION
100
80
60
40
20
0
20
40
60
80
100
120
140
160
PO_2

Fig. 3

C. Protein-PVP Interaction:

Burstein[3] has reported the precipitation of β-lipoprotein from plasma on addition of PVP and has suggested this be used as a preparative procedure for separating β-lipoprotein. The concentration of PVP used to protect red cells is high enough to cause this precipitation, and we have observed it in our studies as a flocculant material in the supernatant plasma when whole blood containing PVP is centrifuged. It was formed when the PVP concentration was greater than 4%. The precipitate is readily soluble in isotonic saline and in plasma: one volume dissolves in six volumes of saline or plasma.

To get an indication of possible harmful effects of the precipitate on infusion of blood containing it, β-lipoprotein was precipitated from 150 cc. of rabbit plasma (over one blood volume) and infused. There were no apparent effects as indicated by gross behavior of the animal.

Because infusion of a whole blood preparation containing β-lipoprotein in suspension might be considered harmful, several ways of eliminating the problem have been examined. One was to separate plasma from cells and treat the plasma with PVP. The precipitate could be removed by centrifugation and the plasma with added PVP used to resuspend the red cells, these having been washed with saline to remove trapped plasma.

The other way was to separate red cells from plasma and resuspend in a medium containing PVP. This procedure is described in a later section.

D. Evaluation of Processing Parameters:

When extra-cellular, protective additives, such as PVP, are used, stringent control of processing conditions is needed to give the highest recovery of stable cells in reproducible fashion. An important part of recent studies has been the determination of optimum processing conditions and the degree of variability permitted, using *in vivo* survival as the measure of stability. As was pointed out in an earlier section, *in vitro* tests are not as definitive as is desired in establishing stability of processed cells.

1. PVP Concentration:

Previous studies had shown the minimum concentration of PVP which gives high stability of red cells to be 7 g. per 100 ml.

final red cell suspension. This corresponds to a concentration in the suspending medium of about 10.5 g. per 100 ml. Variation in the freezing and thawing conditions have not resulted in the use of a lower concentration.

2. PVP Molecular Weight:

All recent preparations evaluated by clinical assay have been made with Plasdone C, K-30 PVP. This has an average molecular weight of 40,000. We have established that PVP K-25 (from Light and Co.) and PVP K-22 (from Antara Products), both of about 25,000 average molecular weight, give cells of comparable stability as measured by in vitro tests (Table V).

TABLE V

In Vitro Stabilities of Red Cells

Protected by Various PVP's

PVP	Post-Thaw Recovery	Saline Resuspension Recovery
Plasdone C (avg. mol. wt. - 40,000)	96.3	90.3
K-22 (Antara) (avg. mol. wt. - ~24,500)	96.4	87.8
K-25 (Light and Co.) (avg. mol. wt. - 24,500)	96.2	88.8

PVP was present in whole blood-ACD at a concentration of 7% w/v. Fifty milliliter samples were processed under identical conditions.

3. Freezing Conditions:

Strict control of the freezing conditions continues to be necessary for reproducible attainment of high recovery of stable cells. Immersion of blood containers in a 500-centipoise solution of PVP K-30 in methanol gives a coating that results in optimal heat transfer. Faster or slower cooling than is obtained with this mixture gives poorer results. Shaking in liquid nitrogen during freezing to give a shell of frozen blood has proved to be necessary, for in no test without shaking was a high recovery of stable cells achieved.

4. Thawing Conditions:

Shaking of a container of frozen blood in a warm water bath to thaw it has continued to give best results. The criticality of the parameters of the thawing procedure was shown by the decreased time required for thawing when a new bracket was designed which permits freer access of the warm water to the container; the stability of cells as measured by resuspension in isotonic saline was increased.

5. Collection of Blood:

Blood has been collected in glass bottles or plastic packs and then transferred to the metal container or collected directly into the metal container without apparent difference in results. Blood has been added to a mixture of ACD and PVP, or PVP has been added to blood and ACD, again without noticeable difference.

There have been differences noted among the recoveries and osmotic stabilities after freezing and thawing of bloods collected by the Buffalo Chapter, American Red Cross, the Chronic Disease Research Institute, and the VA Hospital, Buffalo (Table VI). There has been no apparent difference in the techniques of collection. It is tentatively hypothesized that the difference results from the time of collection and, more likely, from the fasting requested of the Red Cross donors. The first clinical test of blood from donors who have fasted indicates this may be a factor in getting a somewhat more stable cell.

TABLE VI

Variation of Red Cell Stabilities

Among Collection Sites

Collection Site	Post-Thaw Recovery	Saline Resuspension Recovery
Buffalo Chapter, American Red Cross	97.2	88.2
Chronic Disease Research Institute	96.1	84.5
Veterans Administration Hospital, Buffalo	95.2	81.7

Pints (420-ml. blood + 160-ml. ACD-PVP) were collected, frozen, and thawed under identical conditions.

IV. PVP-HUMAN SERUM ALBUMIN:

The presence of insoluble β-lipoprotein in suspensions of cells containing plasma and PVP led to test of cell suspensions containing less, or no, plasma. Blood was centrifuged to pack the red cells and the supernatant plasma removed. Red cells were then suspended in a medium containing PVP and albumin. Because we had observed, when testing serum albumin as a protective material, that it increased the protective action of PVP when red cells were processed in a solution containing both, we have concentrated on this mixture.

Processing involves separation of red cells from plasma, washing by resuspension of the cells in isotonic saline when the plasma is to be substantially completely removed; separation of the red cells from wash solution and resuspension in a solution containing PVP and albumin.

A. Description of Process:

Blood (450 ml.) is collected into 72 to 75 ml. of ACD-A and then centrifuged. After separation of the plasma from the cells, they are resuspended in an equal volume of additive containing 14% Plasdone C, 3% human serum albumin, and 0.6% sodium chloride. The suspension is frozen in the same way as whole blood + PVP.

B. In Vitro Stability:

Using the same criteria of stability as those described in the section on Whole Blood Plus PVP it was found that red cells suspended in a medium containing 11% PVP and 2.5% serum albumin were of good stability and that these concentrations were the minimum that resulted in maximum stability of frozen and thawed cells. With them it was possible to get 97% recovery of intact cells after thawing and 90% recovery of cells on resuspension 1/40 in isotonic saline.

C. In Vivo Stability:

Assay of survival of cells following infusion showed the cells to be more stable than those recovered from whole blood plus PVP. The loss of cells 24 hr. after transfusion was only 12%, in contrast to the 20% loss of cells from whole blood plus PVP (Table VII). This compares favorably with the survival (93 to 95%) of fresh cells that have not been frozen and thawed.

TABLE VII

PVP-Albumin Process

Amount Processed	Trials	Recovery	Survival 30 Min.	24 Hr.	48 Hr.	72 Hr.
1/2 pint	47	97.1±0.3	87±6	88±7	82±7	78±7
pint	5	97.3±0.3	-	89±5	83±5	78±7

Blood collected into ACD-A. Cells separated and resuspended in 14% PVP, 3% albumin and 0.6% NaCl. Thawed cells separated from PVP solution and resuspended in autologous plasma for transfusion.

D. Processing Conditions:

The same freezing and thawing conditions are used for this preparation as for whole blood-PVP after red cells are resuspended in PVP-albumin.

V. PVP ANALYSES FOR PROFESSOR MARK HAYES:

Several procedures for analyzing serum and urine for their PVP contents have been tested. The procedures will be described in detail in a separate report. One of them, involving infrared determination of PVP, has been used to analyze 500 samples of dog serum and urine for Professor Mark Hayes, Yale University School of Medicine. The samples were obtained in the course of a study supported by ONR of the excretion of PVP and its effect on circulatory and renal dynamics of dogs.

VI. BLOOD PROCESSING UNIT FOR THE CENTRAL LABORATORY OF THE NETHERLANDS RED CROSS:

A blood freezing and thawing unit was constructed for and shipped to the Netherlands Red Cross to be used in the processing of red cells separated from plasma. The unit incorporated modifications that had proved desirable from months of operation of earlier units.

Design and construction were carried out to fulfill an agreement with the Netherlands Red Cross that had been authorized by ONR.

VII. REFERENCES:

1 Technical Methods and Procedures of the American Association of Blood Banks, p. 61, Revised Ed., 1960, Chicago, Ill.

2 Pranker, J. Physiol. 143, 325 (1958).

3 Burstein, M., Compt. rend. acad. sci., 244, 3189 (1957).